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Effects of Vaccination with a Mesogenic Viral Strain against Gumboro Disease on the *in vitro* Lymphocyte Activity in Broiler Chickens

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Abstract. Testing the effect of a mesogenic virus strain on lymphocytes from broiler chickens, vaccinated against infectious bursal disease was performed by *in vitro* blast transformation of lymphocytes. Peripheral blood was collected on anticoagulant 24 hours prior to vaccination, as well as 7 and 14 days after the vaccination. Blood was diluted 1:4 with RPMI 1640, then distributed in 96 plates in duplicate for each experimental variant (control, phytohemagglutinin - PHA, concanavalin A - ConA, lipopolysaccharide - LPS). After 48 hours of incubation at 5% CO2, cell growth was estimated by spcetrophotometry of residual glucose (ortho-toluidin method). Blastization indices were calculated (%). The used vaccine strain decreased the ability of post vaccination *in vitro* lymphocytes transformation. Thus, the first week after vaccination, the inhibitory effect was higher for B lymphocytes when compared to T lymphocytes, while after two weeks following the vaccination, the degree of inhibition was non significant.

Keywords: vaccination, infectious bursal disease, blast transformation, lymphocytes.

INTRODUCTION

Vaccinating chickens against infectious bursal disease was and still is a major concern for specialists in the field of avian pathology. Strains with different virulence were used for vaccination and no direct relationship between the quality of vaccine and immune response was found. Moreover, it was found that pathogenic strains induce high level of bursa Fabricii lesions, comparable to those occurring in acute episodes (Morales *et al*. 2004), while those with lower pathogenicity cause similar lesions to those of subclinical episodes. These changes, depending on the seriousness of their quality directly affect the postvaccinal humoral and cell- mediated immune responses. During *in ovo* vaccination, the most severe lesions occur on days 4-5 postvaccination (Rautenschlein and Haas, 2005). In case of post eclosion vaccination, depending on the vaccinal strain used, the most severe injuries can occur between three and seven days after vaccination (Ezeokoli *et al*.1990) or later, after approximately 17 days post vaccination. The changes that occur following the vaccination are partly reversible, depending on immune status, genetic resistance of chickens and especially depending on the vaccine strain and vaccination protocol (Rautenschlein and Haas, 2005). In some cases, the appearance of new antigenic variants, some with high pathogenicity, especially in vaccinated herds was observed (Muller *et al*. 2003), which further complicated the prophylaxis of infectious bursitis. In this study were tested the effect of a mesogenic vaccine strain on the *in vitro* blastization ability of major classes of lymphocytes derived from vaccinated broiler chicken.

MATERIAL AND METHODS

The biological material consisted of 30 broiler chicken, subject of intensive farming of 42 days. The farm premises consisted of 8 halls with 18,000 chickens per hall. The chickens were kept on the ground on a bedding of wood shavings. Ventilation, temperature, light and humidity were electronically controlled. The watering system was represented by dripping with a spoon; while feeding was automatic. The fooder was purchased from a specialized company, according to the age group: prestarter (24% protein), 1 starter (22% protein), 2 starter (20% protein) and finisher (18% protein). Chickens were given vitamin and mineral supplements between 6 and 11 respectively 15 and 21 days of life. During the first 21 days, chickens were given coccidiostatic medicated fodder and two treatments with antibiotics for 5 days each (from day 1 with amoxycillin and day 18 with enrophloxacyn). The halls were populated with day-old chicks vaccinated against infectious bursitis and Newcastle disease. The birds were vaccinated against Newcastle disease on day 9, against Gumboro disease with an intermediate strain, on day 12 days, and against Newcastle disease by aerosolization on day 21. Blood was collected on heparin (50 IU / ml), 24 hours prior to vaccination (sampling 1), 7 (sampling 2) and 14 days post vaccination (dpi) (sampling 3) to monitor the *in vitro* effect of the vaccine strain on T and B lymphocytes. Blood was diluted 1:4 with RPMI 1640 (5% fetal calf serum, 1000 IU of penicillin and 1000 μg of streptomicin / ml, pH 7.2) and distributed in 96 well plates in duplicate for each experimental variant, same for each of the samplings:

-blood + M = medium
-blood + medium+ phytohemagglutinin (mitogenic specific T lymphocytes) = PHA
-blood + medium + concanavalin A (mitogenic specific T lymphocytes) = ConA
-blood + medium + lipopolysaccharide (mitogenic specific B lymphocytes) = LPS

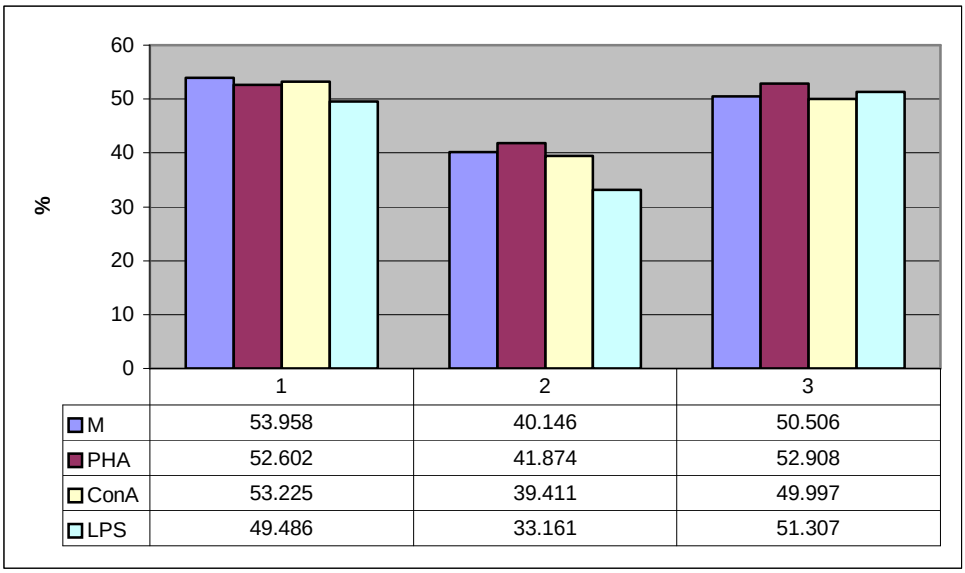
After 48 hours of incubation at 5% CO_2, cell growth was estimated by determining the residual glucose by spectrophotometry (ortho-toluidin colorimetric method). Blast transformation indices were calculated (%). Statitical significance of the data was estimated by Student t-test.

RESULTS AND DISCUSSIONS

The average values of blastization indices were presented in fig 1. At 7 dpi a decreased ability of lymphocytes to spontaneously transform was observed, while after 7 more days (14 dpi) a weaker capacity of cells to grow, without reaching the levels before vaccination was encountered. When the specific mitogens for T lymphocytes were added, a different degree of reception of the mitogenic stimulus was observed, depending on its chemical composition. Thus, the index in vaccinated birds before the co-cultivation with the mitogenic stimulus was higher for ConA, attaining almost the value of the spontaneous blast transformation index. At the second sampling (7 dpi), the suppressive effect of the vaccination was obvious for all the cultural variants. As hypothesized, the B lymphocytes, originating from the bursa, target to the vaccine, were mostly affected. PHA still exerted its mitogenic capacity, when compared to the control, but it was statistically insignificant. Blastization index values both at 7 and 14 dpi were higher for PHA, when compared to control cultures and to ConA. In what concerns the stimulation of B lymphocytes, the reception of the mitogenic stimulus before vaccination was low, blastization index having the lowest value. At 7 dpi, the ability of blastization of B lymphocytes remains low, with the lowest value in comparison to other experimental variants.

These data suggested an improvement of blastization capacity and a better degree of reception of the mitogenic stimuli, to almost the extent attained before the vaccination.

The spontaneous blastization index (M) was at 7 dpi of only 74.4% compared to the index before the vaccination. The functional disability induces by the vaccine was present within the spontaneous mitogenesis process as well, the index reaching only 93.6% of the index noticed before the antigen priming of the birds. There was a 0.97% decrease of the PHA M induces stimulation index when compared to the spontaneous one, before the vaccination of the birds, while for LPS it was only of 0.09% (ConA variant).



	1	2	3
M	53.958	40.146	50.506
PHA	52.602	41.874	52.908
ConA	53.225	39.411	49.997
LPS	49.486	33.161	51.307

Figure 1 – Dynamics of blast transformation indices in the experimental variants

The initial value of blastization index for ConA was higher by 1.01% when compared to that of PHA.

After two weeks following the vaccination, the situation was different, the observed differences were higher in reception of the various mitogenic stimuli. Thus, PHA had a stimulating effect (100.28%) while ConA induced an inhibitory effect (93.95%), when compared to the spontaneous index of the sampling. Initial values of blastization index of B lymphocytes induced by LPS represent 91.71% when compared to M (control). After seven days, the suppression exerted by the vaccinal virus was more pronounced, the growth of B lymphocytes attaining only 67.01% of its initial value. The blast transformation capacity of B lymphocytes was stimulate by two weeks after the vaccination, increasing to 101.58% of the initial value.

The viral strain used for vaccination induces after 7 days post vaccination an intense suppression of the functional capacity of both T and B lymphocytes, as indicated by the low percentages of stimulation obtained for all the mitogens, when compared to the spontaneous pre vaccination index (77% for PHA, 73.04% for ConA and 61.45% for LPS). At 14 dpi degree of inhibition decreased, the blastization index for PHA and ConA being of 98.05% and 92.65% respectively while that for LPS was of 95.08%. These data are in correlation with the data from literature, infectious bursitis virus inhibiting the ability of blast transformation of lymphocytes from peripheral blood (Confer *et al*.,1981; Saif, 2001). After vaccination, the used vaccine strain caused a decrease in the *in vitro* ability of lymphocytes to form blasts. In the first week after vaccination, inhibitory effect was higher for B lymphocytes, when compared to T subpopulations. In two weeks pi the lymphocytes recovered, responding to

mitogens, but being somewhat suppressed in exerting their spontaneous mitogenic capacity. The short recovery period of the lymphocytes after the antigen priming stress indicated Because vaccine strain has inhibitory effect on the ability of blastization of lymphocytes in that this vaccine strain could be successfully used in protecting the chickens against Gumboro disease during their economic lifespan.

CONCLUSIONS

The inhibitory effect of the mesogenic vaccinal strain of infectious bursal disease virus was higher in B lymphocytes than in T lymphocytes. The degree of reception of the mitogenic stimulus for T lymphocytes was dependent on the chemical composition therefore ConA had a higher inhibitory effect than PHA. The inhibitory effect of the vaccine gradually diminished so that by day 14 after vaccination, the blast stimulation index of lymphocytes returned to values close to those encountered before the vaccination.

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